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(54) DNA and polypeptide for tumor-associated antigen CO-029

DNS und Polypeptide von tumorassoziiertem Antigen CO-029

ADN et polypeptide de l'antigène CO-029 associé à une tumeur

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- **PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA, vol. 87, September 1990, Washington, DC (US); S. SZALA et al., pp. 6833-6837**
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- **PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA, vol. 87, May 1990, Washington, DC (US); S. SZALA et al., pp. 3542-3546**
- **CANCER RESEARCH, vol. 48, no. 11, 01 June 1988, Philadelphia, PA (US); H. HOTTA et al., pp. 2955-2962**

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EP 0 478 146 B1

Description

The U.S. Government retains some rights in this invention which was made under grant CA21124-11 from the National Institutes of Health.

BACKGROUND OF THE INVENTION

A number of monoclonal antibodies (mAb) have been derived from the immunization of mice with human gastrointestinal tumor cell lines (Koprowski, et al., (1979), Somat. Cell Genet. vol. 5, pp. 957-972; Steplewski, et al., (1982) in Methods in Cancer Research, eds. Busch, et al., (Academic, New York), vol. XX, pp: 285-316; Herlyn, et al., (1984), J. Immunol. Meth., vol. 73, pp. 157-167; Gottlinger, et al., (1986), Int. J. Cancer, vol. 38, pp. 47-53; Girardet, et al., (1986), J. Immunol., vol. 136, pp. 1497-1503). Investigations into the antigenic structures recognized by these mAb have identified a group of glycolipid and glycoprotein antigens (Steplewski, et al., (1982) in Methods in Cancer Research, eds. Busch, et al., (Academic, New York), vol. XX, pp. 285-316). The 40 kDa cell surface glycoprotein (Ross, et al., (1986), Hybridoma, vol. 5, pp. S21-S27) recognized by mAb CO17-1A (Herlyn, et al., (1986), Hybridoma, vol. 5, pp. S3-S8), and several other independently derived mAb (Gottlinger, et al., (1986), Int. J. Cancer, vol. 38, pp. 47-53; Girardet, et al., (1986), J. Immunol., vol. 136, pp. 1497-1503; Ross, et al., (1986), Biochem. Biophys. Res. Comm., vol. 135, pp. 297-303), is one of the most well-characterized tumor-associated antigens. Another cell surface glycoprotein antigen, detined by mAb CO-029 (formerly called 1116NS-29) (Koprowski, et al., (1979), Somat. Cell Genet. vol. 5, pp. 957-972), has been shown to be a ~32 kDa monomer (Sela, et al., (1989), Hybridoma, vol. 8, pp. 481-491). The CO-029 antigen was found to be expressed on gastric, colon, rectal, and pancreatic carcinomas, but not on most normal tissues (Sela, et al., (1989), Hybridoma, vol. 8, pp. 481-491). MAb CO-029 has been shown to mediate antibody-dependent cell-mediated cytotoxicity in vitro (Koprowski, et al., (1979), Somat. Cell Genet. vol. 5, pp. 957-972). However, the CO-029 antigen has not been structurally characterized, nor has its coding sequence been isolated.

The recent molecular cloning of cDNA for tumor-associated antigens has provided information about antigen structure and the evolution of the genes encoding these antigens. cDNA clones have been isolated (Szala, et al., (1990), Proc. Natl. Acad. Sci. USA, vol. 87, pp. 3542-3546) for the GA 733-2 carcinoma-associated antigen defined by mAB GA733 (Herlyn, et al., (1986), Hybridoma, vol. 5, pp. S21-S27). Transfection experiments with a GA733-2 cDNA clone have shown that it encodes both the GA733 and CO17-1A epitopes (Szala, et al., (1990), Proc. Natl. Acad. Sci. USA, vol. 87, pp. 3542-3546). Sequence analysis of GA733-2 found it to be identical with the mAb defined, tumor-associated antigen KSA (Strand, et al., (1989), Cancer Res., vol. 49, pp. 314-317). A related gene, GA733-1, has been identified and was found to have a 50% amino acid sequence homology with the GA733-2 antigen (Szala, et al., (1990), Proc. Natl. Acad. Sci. USA, vol. 87, pp. 3542-3546; Linnenbach, et al., (1989), Proc. Natl. Acad. Sci. USA, vol. 86, pp. 27-31). GA733-1 is an intronless gene that was shown to be transcribed at high level in pancreatic carcinoma cell lines (Linnenbach, et al., (1989), Proc. Natl. Acad. Sci. USA, vol. 86, pp. 27-31). The GA733 family of antigens are type I transmembrane proteins of unknown function.

There is a need in the art for additional cloned tumor-associated antigens. The clones can be used to determine nucleic acid sequences as well as to produce preparations of antigens which may be useful as vaccines to stimulate anti-tumor antibody production in cancer patients.

SUMMARY OF THE INVENTION

It is an object of the invention to provide DNA molecules encoding tumor-associated antigen CO-029.

It is another object of the invention to provide DNA molecules which can be used to express antigen CO-029 in cultured cells.

It is yet another object of the invention to provide sequence analysis of the CO-029 antigen to determine the antigenically important portions of the molecule.

It is an object of the invention to provide polypeptides which consist essentially of the extracellular domains of tumor-associated antigen CO-029.

It is another object of the invention to provide polypeptides which are immunoreactive with CO-029.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment an intron-free DNA molecule is provided which encodes a tumor-associated antigen, said antigen immunoreactive with monoclonal antibody CO-029.

In another embodiment a polypeptide is provided consisting essentially of an extracellular domain of CO-029.

In still another embodiment a preparation of CO-029 antigen is provided which is free of other human proteins.

In yet another embodiment of the invention transformed cells are provided which contain DNA molecules encoding CO-029 antigen.

The and other embodiments of the invention which will be described in more detail below, provide the art with an additional molecularly, cloned tumor-associated antigen. This allows the unlimited production of the antigen in cell which are not malignant and the potential use of the antigen as a biological response modifier to stimulate the immune systems of human cancer patients to combat colorectal, lung and other carcinomas as well as astrocytomas.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the detection of the CO-029 antigen in transfected COS by mixed hemadsorption assay (MHA). Cells were incubated with mAb CO-029, and then reacted with an indicator system consisting of sheep erythrocytes treated with mouse antiserum against sheep erythrocytes and goat anti-mouse IgG. (A) Untreated COS cells. (B) COS cell 3 days after transfection with CO-029-5 DNA.

Figure 2 shows Western blot detection of the CO-029 antigen in transfected COS cell. Total cell extracts were derived from control SW948 colorectal carcinoma cell (lane 1), untreated COS cells (lane 2), and COS cells 3 days after transfection with CO-029-5 DNA (lane 3).

Figure 3 shows (A) the sequence of full-length CO-029 cDNA with predicted amino acid sequence. The single-letter amino acid code is used. The positions of 12 Cysteine residues (circles), a potential N-linked glycosylation site (dotted overline), and 4 hydrophobic regions (bold overline) are indicated. Consensus sequences for initiation of translation (underline), mRNA turnover (overline) and poly(A) addition (dotted underline) are shown. (Panel B) shows a Kyte-Doolittle hydrophobicity plot of the CO-029 antigen showing the 4 hydrophobic regions.

Figure 4 shows alignment of CO-029 related sequences. The CO-029, ME491; and Sm23 sequences are shown in their entirety; the homologous portion of the CD37 sequence is presented. The single-letter amino acid code is used. Conserned Cysteine residues are in boldface type. Potential N-linked glycosylation sites (underline); and predicted transmembrane (Tm), extracellular (Ex) and cytoplasmic (Cy) domains are illustrated, Gaps (....) were inserted to maximize the alignment; a consensus sequence (Cons.) was calculated.

Figure 5 shows models for the CO-029 family of membrane antigens. (A) The CO-029, ME491, and Sm23 antigens. (B) The CD37 antigen.

Figure 6 shows in panel A a Northern blot analysis with the CO-029-5 DNA probe. mRNA was derived from the SW948 colorectal carcinoma cell line (lane 1), the SW707 rectal carcinoma cell line (lane 2), the pancreatic carcinoma cell lines Capan-2 (lane 3) and BXPC-3 (lane 4), the melanoma cell lines WM1158 (lane 5) and WM35 (lane 6). In panel B hybridization was performed with a control probe from the enolase gene.

Figure 7 shows the binding of monoclonal antibodies (mAbs) ME491 and CO-029 in radioimmunoassay (RIA) to a panel of human tumor cell lines. Results are presented after subtraction of non-specific mAb P3 binding (typically 100-200 cpm).

DETAILED DESCRIPTION

An intron-free DNA molecule has been cloned and analyzed which encodes the tumor-associated antigen CO-029. When the DNA molecule is transfected into COS cell which do not express the antigen, the resulting transfectants synthesize antigen bearing the CO-029 epitope (as defined by the monoclonal antibody CO-029.) The antigen expressed in transfected COS cell is indistinguishable by Western blotting from that expressed in cell of a colorectal cell line.

DNA sequencing of the cloned DNA molecule reveal an antigen with a protein molecular weight of 26,044. This implies that up to 8 kDa of the native glycoprotein antigen may be due to glycosylation. The complete nucleic acid and predicted amino acid sequences are shown in Figure 3.

DNA molecules and polypeptides according to the present invention may contain all or part of the sequences shown in Figure 3. Desirably polypeptides retain the ability to bind to antibodies which are immunoreactive with the extracellular domains of CO-029. Such binding can be readily tested by raising antibodies against a particular polypeptide. If the antibodies immunoreact with intact colorectal carcinoma cells which express the antigen on they cell surfaces, the polypeptide represents an extracellular epitope. As taught herein, one such extracellular domain comprises amino acids numbered 34 to 57, and another comprises amino acids 110 to 205. In one embodiment of the invention the polypeptide is immunoreactive with monoclonal antibody CO-029. In another embodiment of the invention a polypeptide is covalently linked to an amino acid sequence which causes the polypeptide to be secreted from cell in which it is synthesized. Such amino acid sequences, signal sequences, are well known in the art.

Polypeptides of the present invention may be free of glycosylation. Such glycosylation-free polypeptides can be synthesized for example in a non-glycosylating microorganism such as E. coli, or in the presence of tunicamycin in cell which do glycosylate mammalian proteins. Alternatively the polypeptides can be chemically synthesized, for example using an automatic synthesizer, according to the disclosed sequence. Glycosylated polypeptides can be made in mammalian and other higher eukaryotic cell.

Because the polypeptides of the invention can be made by recombinant DNA-containing organisms or cell or synthetically, they can be entirely free of other human proteins. In contrast, native antigens isolated from human cell generally do not achieve such a level of purity. Since the polypeptide need not be isolated from malignant human cells, there is reduced risk associated with administration of the polypeptide to humans, for example, as an injectible.

The polypeptides of the present invention can be used to raise new anti-tumor antibodies. It may be desirable to isolate antibodies which are reactive with other epitopes of the CO-029 antigen than the one defined by monoclonal antibody CO-029. Alternatively, it may be desirable to isolate antibodies which react with the same epitope but have different properties from CO-029, such

as enhanced or reduced cytotoxicity. The polypeptides can also be used as mentioned above, as injectibles. They can be used as biological response modifiers to stimulate a cancer patient's immune system to attack tumors expressing the CO-029 antigen.

The polypeptides of the invention may be coupled to larger moieties, such as keyhole limpet hemocyanin, to enhance their immunogenicity. They can also be mixed with adjuvants, as is known in the art. In one particular embodiment the polypeptide is mixed with viral proteins of vaccinia. This is conveniently achieved by forming recombinant vaccinia virus genomes which express the CO-029-related polypeptide. The recombinant virus can be grown in baby hamster kidney cells (BHK) for example, and the recombinant virus injected into humans. The polypeptides of the present invention can also be mixed with other bioactive or immunologically active polypeptides.

Polypeptides of the invention may also be used diagnostically to quantitate the amount of antigen present in a biological sample. For example, defined amounts of polypeptide can be used in competitive binding assays with antibodies directed against the polypeptide.

The DNA molecules of the present invention can be used to express the antigens of the invention. The DNA molecules are used to transform suitable human or non-human cells, such as mouse, monkey, yeast, etc. Methods of transforming and propagating such cells in culture are well known. In one embodiment the antigen-coding DNA molecule is linked to a vector which can replicate in E. coli. Such vectors are well-known in the art. In another embodiment the DNA molecule causes COS cell to express a protein which is immunoreactive with monoclonal antibody CO-029. Such expression may be stable or transient, depending on the vector used for transfection. Portions of the CO-029 gene can be isolated using the polymerase chain reaction (PCR). For example, the portion of the gene encoding the large extracellular domain can be amplified and isolated using primers designed from the disclosed sequence. This can be ligated to DNA encoding a signal sequence so that when expressed in a recombinant cell the extracellular domain is expressed and secreted from the cell. Suitable signal sequences are known in the art.

DNA molecules can also be used as probes and primers in polymerase chain reactions or hybridization studies to quantitate expression of the CO-029 mRNA in a biological sample. Such quantitation may provide diagnostic or prognostic information on a patient's disease. Thus the DNA molecules of the invention may be as small as about 10 nucleotides and be useful as probes or primers, if they hybridize specifically to the CO-029 gene. Some DNA molecules of the invention are only as large as is necessary to encode a polypeptide which is immunoreactive with antibodies raised using the entire CO-029 antigen as an immunogen.

The following examples illustrate further aspects of the invention but do not limit the invention to these particular embodiments.

Examples

Example 1. **Immunoselection of cDNA Clones.** A cDNA library (Szala, et al. (1990), Proc. Natl. Acad. Sci. USA, vol. 87, pp. 3542-3546) prepared from the SW948 colorectal carcinoma cell line was transfected into COS cells by the DEAE-dextran method (Seed, et al. (1987), Proc. Natl. Acad. Sci. USA, vol. 84, pp. 3365-3369). COS cells transiently expressing antigen were selected by "panning Natl." (Seed, et al. (1987), Proc. Acad. Sci. USA, vol. 84, pp. 3365-3369) with mAb CO-029. Episomal DNA was recovered from the panned cells and transferred into Escherichia coli MC1061/P3 cells. Recombinant DNA was isolated from the E. coli cells and further enriched for CO-029 sequences by two additional cycles of immunoselection, with COS cells (African Green Monkey Kidney Cells) transfections being performed by spheroplast fusion (Seed, et al. (1987), Proc. Natl. Acad. Sci. USA, vol. 84, pp. 3365-3369) instead of DEAE-dextran. The first immunoselection of the total cDNA library yielded 11,600 drug resistant bacterial colonies; 600 and 2900 colonies were observed after the second and third selections, respectively.

Example 2. **Analysis of Immunoselected Clones.** Ten drug-resistant bacterial colonie resulting from the final selection were chosen at random and analyzed for cDNA insert size by digestion at flanking Xhol sites. Six out of 10 clones contained cDNA inserts 1.1 kb in size. DNA was prepared from a 1.5 ml bacterial culture of one of the clones, CO-029-5. One-fifth of this DNA was introduced into COS cells by the DEAE-dextran method. After a 3 day transient expression period, the transfectants were analyzed with mAb for the expression of the CO-029 epitope.

Transfected cells were assayed by the mixed hemadsorption assay (MHA) as described by Herlyn, et al. (Cancer Res., (1980), vol. 40, pp. 3602-3609). In MHA using mAb CO-029, -30% of transfected COS cells rosetted (Fig. 1B), whereas untreated COS cells did not react (Fig. 1A).

To confirm this result, Western blot analysis with mAb was performed on COS cells transiently expressing the CO-029-5 clone. For Western blot analysis, cells were lysed in a buffer containing NP-40 (Ross, et al., (1986), Biochem. Biophys. Res. Comm., vol. 135, pp. 297-303), and 40 μg aliquots of non-reduced, non-heated, total protein were electrophoresed on a 12% SDS-polyacrylamide gel (Laemmli, (1970), Nature (London), vol. 227, pp. 680-685). Proteins were electroblotted onto a nitrocellulose filter, which was incubated with affinity purified CO-029 mAb at 1 μg/ml, and then with affinity purified goat anti-mouse IgG alkaline phosphatase conjugate and substrates for color development (Promega Biotech, Madison, WI).

Transfected cells expressed a 27-34 kDa antigen (Fig. 2, lane 3) characteristic of the native CO-029 anti-

gen expressed in control SW948 cells (Fig. 2, lane 1). Both cell types expressed several discrete forms of CO-029 between 27 and 34 kDa. Antigen was not detected in untreated COS cells (Fig. 2, lane 2). Thus, the CO-029-5 clone encoded the epitope recognized by the CO-029 mAb.

Example 3. **DNA Sequence Determination and Analysis.** Both strands of the full-length cDNA clone CO-029-5 were sequenced by the dideoxynucleotide method (Sanger, et al. (1977), Proc. Natl. Acad. Sci. USA, vol. 74, pp. 5463-5467). Sequence reactions were performed with T7 DNA polymerase (Pharmacia, Piscataway, NJ) using vector-specific and CO-029-specific primers. Compressions were resolved by substituting 7-deaza dGTP for dGTP.

The 1.1 kb sequence of CO-029-5 revealed an open reading frame for 237 amino acids beginning at the 5′-proximal ATG codon, which was found to be flanked by sequences similar to the consensus sequence for initiation of translation (Kozak, (1987), Nucleic Acids Res., vol. 15, pp. 8125-8147) (Fig. 3A). A protein molecular weight of 26,044 is predicted for CO-029. Thus, up to 8 kDa of the 27-34 kDa CO-029 glycoprotein may be accounted for by glycosylation (Sela, et al. (1989), Hybridoma, vol. 8, pp. 481-491). A single potential N-linked glycosylation site was observed (Fig. 3A). The 5′ untranslated region consists of 138 residues; the 232 base 3′ untranslated region contained one consensus sequence for poly(A) addition and two sequences for mRNA turnover (Shaw, et al., (1986), Cell, vol. 46, pp. 659-667) (Fig. 3A).

Analysis of the distribution of hydrophobic and hydrophilic amino acids (Kyte, et al., (1982), J. Mol. Biol., vol. 157, pp. 105-132) (Fig. 3B) suggested that CO-029 is a type III integral transmembrane protein (Singer, et al., (1987), Proc. Natl. Acad. Sci. USA, vol. 84, pp. 1960-1964) Four hydrophobic stretches of -25 amino acids were found to be separated by hydrophilic amino acids (Fig. 3A and B).

The CO-029 sequence was tested for homology by searching release 63 of GenBank with the program TFASTA (Pearson, et al. (1988), Proc. Natl. Acad. Sci. USA, vol. 85, pp. 2444-2448), and release 23 of the NBRF-protein database with the programs FASTP (Lipman, et al. (1985), Science, vol. 227, pp. 1435-1441) and FASTA (Pearson, et al. (1988), Proc. Natl. Acad. Sci. USA, vol. 85, pp. 2444-2448). Sequences with optimized scores >100 were further studied with the programs ALIGN (Dayhoff, et al., (1983) in Methods in Enzym., eds. Hirs, et al., (Academic, New York), vol. 91, pp. 524-545) and LINE-UP (Devereux, et al., (1984), Nucleic Acids Res., vol. 12, pp. 387-395).

Database analysis revealed that the CO-029 antigen was related to the human melanoma-associated antigen ME491 (Hotta, et al., (1988), Cancer Res., vol. 48, pp. 2955-2962) and the human leukocyte antigen CD37 (Classon, et al., (1989), J. Exp. Med., vol. 169, pp. 1497-1502). Other investigators have found ME491 to be homologous to the Sm23 antigen of the parasitic helminth S. mansoni (Wright, et al. (1990), J. Immunol., vol. 144, pp. 3195-3200). Analysis ot these sequences with the program ALIGN indicated that these homologies were statistically significant. For example, pairwise comparison of the CO-029 sequence with the ME491, Sm23, and CD37 sequences, using a gap penalty of 40, resulted in alignment scores 34, 30, and 18 SD units above the mean score of 100 random runs, respectively.

A multiple sequence alignment indicated that the positions of Cys and Gly residues were particularly well conserved in the CO-029 family (Fig. 4). Sequence homology was greatest in transmembrane and cytoplasmic domains predicted by hydrophobicity analysis (Kyte, et al., (1982), J. Mol. Biol., vol. 157, pp. 105-132) (data not shown). This homology was evident even in species as evolutionarily divergent as Homo sapiens and S. mansoni. For example, there were 10 consecutive amino acid identities occurring in CO-029 and Sm23 beginning at residue 74 of the alignment (Fig. 4). In contrast to the transmembrane and cytoplasmic domains, the extracellular domains probably have sustained insertions and/or deletions. The sequences of the predicted extracellular domains were divergent, with the exception of the positions of Cys residues. The number and positions of potential N-linked glycosylation sites varied amongst these antigens, but all positions corresponded to the major hydrophilic domains (Fig. 4).

Two classes of antigen could be distinguished within this family. CO-029, ME491, and Sm23 proteins were found to be approximately equal in length and homologous throughout (Fig. 4). In contrast, the homology of CD37 to CO-029, ME492, and Sm23 was limited to the NH_2-terminal half of these antigens (Fig. 4).

Assuming that the CO-029, ME491, and Sm23 proteins have four transmembrane domains and assuming that the positions of the potential N-linked glycosylation sites (Fig.. 4) place the major hydrophilic domains outside the cell, these antigens would be orientated so that the NH_2 and COOH-terminal ends would be on the cytoplasmic side of the membrane (Fig. 5A). Two extracellular domains, and three short cytoplasmic domains were predicted (Figs. 4 and 5A). The portion of CD37 homologous to the other CO-029-related antigens consisted of three transmembrane and two cytoplasmic domains (Figs. 4 and 5B).

Example 4. **CO-029 Expression in Tumor Cell Lines.** Transcription of the CO-029 gene in human tumor cell lines was analyzed by RNA blotting. Two µg of poly A+ cytoplasmic mRNAs (Berger, et al., (1979), Biochemistry, vol. 18, pp. 5143-5149) were electrophoresed on a 2.2 M formaldehyde/1% agarose gel (Lehrach, et al., (1977), Biochemistry, vol. 16, pp. 4743-4751) and transferred to nitrocellulose. The CO-029-5 cDNA clone was nick-translated and hybridized to the filter in a solution containing 50% deionized formamide and 5 x SSC [20 x SSC is 3.0 M sodium chloride/0.3 M sodium citrate, pH 7.0] at 42°C overnight. The filter was washed at high

stringency in 0.1 x SSC/0.1% SDS at 65°C. The probe was then removed and the filter was hybridized to the control α-enolase cDNA probe (Giallongo, et al., (1986), Proc. Natl. Acad. Sci. USA, vol. 83, pp. 6741-6745).

Transcription of the CO-029 gene was detected in the SW948 colorectal carcinoma and in the SW707 rectal carcinoma and cell lines (Fig. 6A, lanes 1 and 2). The steady state level of CO-029 mRNA differed in there two lines, while the level of α-enolase mRNA was approximately equal (Fig. 6B, lanes 1 and 2). The size of the full-length cDNA clone (Fig. 3A) correlated with the observed 1.15 kb CO-029 transcript. Two pancreatic carcinoma and two melanoma cell lines were negative for CO-029 mRNA (Fig. 6A, lanes 3-6). CO-029-related transcripts were not observed under the high stringency hybridization and washing conditions used here.

Examples 5. **Antigen Expression in Tumor Cell Lines.** [^{3}H] thymidine incorporation was measured after incubation of cell with purified mAbs in chemically defined, serum-free medium. The colorectal carcinoma cell line SW948 and the melanoma cell line WM852 were aliquoted (100μl) to 96-well plates at 1.5 x 10^4 cells/well. Aliquots of mAbs CO-029 and ME491 (100μl and 5-250 μg/ml) were added to each cell type 18 hr after seeding. After 24 hr, each culture was pulsed for 18 hr with 1 μCi of [methyl ^{3}H]thymidine (48 Ci/mmole; 1 Ci=37 GBq). Cells were trypsinized, collected with an automatic cell harvester (Skatron, Sterling, VA), and cell-associated radiactivity was determined in triplicate by liquid scintillation couting.

CO-029 and ME491 antigen expression was compared in different tumor cell lies by RIA. Several cell lies co-expressed these genes (Figure 7). The major qualitative difference in the expression of the two genes was that melanoma cell lines expressed the ME491 antigen but not the CO-029 antigen.

Claims

Claims for the following Contracting States: AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE

1. An intron-free DNA molecule which encodes a tumour-associated antigen, said antigen being immunoreactive with monoclonal antibody CO-029 wherein the amino acid sequence of said antigen is shown in Figure 3.

2. An intron-free DNA molecule according to claim 1, wherein the tumour associated antigen has a protein molecular weight of 26,044.

3. An intron-free DNA molecule according to claim 1 or 2, wherein the nucleic acid sequence of said DNA molecule is shown in Figure 3.

4. An intron-free DNA molecule according to any preceding claim, which is covalently linked to a vector which can replicate in E. coli.

5. An intron-free DNA molecule according to any preceding claim, which causes expression of a protein in COS cells immunoreactive with monoclonal antibody CO-029.

6. An intron-free DNA molecule consisting of a nucleic acid sequence shown in Figure 3.

7. An intron-free DNA molecule which hybridizes to DNA having the sequence shown in Figure 3.

8. An intron-free DNA molecule which codes for an extracellular domain of the CO-029 antigen wherein the amino acid sequence of said antigen is shown in Figure 3.

9. A polypeptide consisting of an extracellular domain of the CO-029 antigen wherein the amino acid sequence of said antigen is shown in Figure 3.

10. A polypeptide according to claim 9, wherein the domain comprises amino acids 34 to 57 of CO-029.

11. A polypeptide according to claim 9, wherein the domain comprises amino acids 110 to 205 of CO-029.

12. A polypeptide according to claim 9, which is immunoreactive with monoclonal antibody CO-029.

13. A polypeptide according to claim 9, which is covalently linked to an amino acid sequence which causes said polypeptide to be secreted from cells.

14. A preparation of CO-029 antigen which is free of other human protein wherein the antigen is made in non-human cells which have been transformed with a DNA molecule according to claim 1 encoding said antigen.

15. Cells transformed with the DNA molecule of claim 1, 6, 7 or 8.

16. Cells according to claim 15 which are non-human.

Claims for the following Contracting State: ES

1. A process for the production of a tumour-associated antigen comprising theuse of an intron-free DNA molecule which encodes a tumour-associated antigen, said antigen being immunoreactive with monoclonal antibody CO-029 wherein the amino acid sequence of said antigen is shown in Figure 3.

2. A process according to claim 1, wherein the tumour associated antigen has a protein molecular weight of 26,044.

3. A process according to claim 1 or 2, wherein the nucleic acid sequence of said DNA molecule is shown in Figure 3.

4. A process according to any previous claim wherein the intron-free DNA molecule is covalently linked to a vector which can replicate in E. coli.

5. A process according to any previous claim wherein the intron-free DNA molecule causes expression of a protein in COS cells immunoreactive with monoclonal antibody CO-029.

6. A process which comprises the use of an intron-free DNA molecule consisting of a nucleic acid sequence shown in Figure 3.

7. A process which comprises the use of an intron-free DNA molecule which hybridizes to DNA having the sequence shown in Figure 3.

8. A process which comprises the use of an intron-free DNA molecule which codes for an extracellular domain of the CO-029 antigen wherein the amino acid sequence of said antigen is shown in Figure 3.

9. A process for the production of a polypeptide consisting of an extracellular domain of the CO-029 antigen wherein the amino acid sequence of said antigen is shown in Figure 3.

10. A process according to claim 9, wherein the domain comprises amino acids 34 to 57 of CO-029.

11. A process according to claim 9, wherein the domain comprises amino acids 110 to 205 of CO-029.

12. A process for the production of a polypeptide according to claim 9, which is immunoreactive with monoclonal antibody CO-029.

13. A process according to claim 9, wherein the polypeptide is covalently linked to an amino acid sequence which causes said polypeptide to be secreted from cells.

14. A process for the production of a preparation of CO-029 antigen which is free of other human protein wherein the antigen is made in non-human cells which have been transformed with a DNA molecule by the process according to claim 1 encoding said antigen.

15. Cells transformed with the DNA molecule of claim 1, 6, 7 or 8.

16. Cells according to claim 15 which are non-human.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten: AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE:

1. Intron-freies DNA-Molekül, welches ein Tumor-assozitertes Antigen codiert, welches Antigen mit dem monoklonalen Antikörper CO-029 eine Immunreaktion eingeht und die in der Fig. 3 gezeigte Aminosäuresequenz aufweist.

2. Intron-freies DNA-Molekül nach Anspruch 1, worin das Tumor-assoziierte Antigen ein Protein-Molekulargewicht von 26.044 besitzt.

3. Intron-freies DNA-Molekül nach Anspruch 1 oder 2. worin die Nucleinsäuresequenz des DNA-Moleküls in der Fig. 3 dargestellt ist.

4. Intron-freies DNA-Molekül nach einem der vorhergehenden Ansprüche, welches kovalent an einen Vektor gebunden ist, der in E. coli zur Replikation fähig ist.

5. Intron-freies DNA-Molekül nach einem der vorhergehenden Ansprüche. welches die Expression eines Proteins in COS-Zellen verursacht, die mit dem monoklonalen Antikörper CO-029 eine Immunreaktion eingehen.

6. Intron-freies DNA-Molekül bestehend aus einer in der Fig. 3 dargestellten Nucleinsäuresequenz.

7. Intron-freies DNA-Molekül das zu DNA hybridisiert, welche die in Fig. 3 dargestellte Sequenz besitzt.

8. Intron-freies DNA-Molekül welches für eine extrazelluläre Domäne des CO-029-Antigens codiert und die Aminosäuresequenz des Antigens in der Fig. 3 dargestellt ist.

9. Polypeptid bestehend aus einer extrazellulären Domäne des CO-029-Antigens, wobei die Aminosäuresequenz des Antigens in der Fig. 3 dargestellt ist.

10. Polypeptid nach Anspruch 9, wonn die Domäne die Aminosäuren 34 bis 57 von CO-029 aufweist.

11. Polypeptid nach Anspruch 9, worin die Domäne die Aminosäuren 110 bis 205 von CO-029 aufweist.

12. Polypeptid nach Anspruch 9, welches mit dem monoklonalen Antikörper CO-029 eine Immunreaktion eingeht.

13. Polypeptld nach Anspruch 9, das kovalent an eine A1ninosäuresequenz gebunden ist, welche die Sekretion des Polypeptids aus Zellen verursacht.

14. Von anderen menschlichen Proteinen freies Präparat des CO-029-Antigens, bei dem das Antigen in nicht-menschlichen Zellen hergestellt worden ist. die mit einem DNA-Molekül nach Anspruch 1, welches das Antigen codiert, transformiert worden sind.

15. Mit dem DNA-Molekül von Anspruch 1, 6, 7 oder 8 transformierte Zellen.

16. Nicht-menschliche Zellen nach Anspruch 15.

Patentansprüche für folgenden Vertragsstaat: ES

1. Verfahren zur Herstellung eines Tumor-assoziierten Antigens umfassenddie Verwendung eines Intron-freien DNA-Moleküls, welches em Tumor-assoziiertes Antigen codiert, welches Antigen mit dem monoklonalen Anttkörper CO-029 eine Immunreaktion eingeht und die in der Fig. 3 gezeigte Aminosäuresequenz aufweist.

2. Verfahren nach Anspruch 1, worin das Tumor-assoziierte Antigen ein Protein-Molekulargewicht von 26.044 besitzt.

3. Verfahren nach Anspruch 1 oder 2, worin die Nucleinsäuresequenz des DNA-Moleküls in der Fig. 3 dargestellt ist.

4. Verfahren nach einem der vorhergehenden Ansprüche, worin das Intronfreie DNA-Molekül kovalent an einen Vektor gebunden ist. der in E-coli zur Replikation fähig ist.

5. Verfahren nach einem der vorhergehenden Ansprüche, worin das Intronfreie DNA-Molekül die Expression eines Proteins in COS-zellen verursacht, welches mit dem monoklonalen Antikörper CO-029 eine Immunreaktion eingeht.

6. Verfahren, welches darin besteht, ein Intron-freies DNA-Molekül zu verwenden, welches aus der in der Fig. 3 dargestellten Nucleinsäuresequenz besteht.

7. Verfahren, welches die Verwendung eines Intron-freien DNA-Moleküls umfaßt, welches zu DNA mit der in der Fig. 3 dargestellten Sequenz hybridisiert.

8. Verfahren, welches die Verwendung eines Intron-freien DNA-Moleküls umfaßt, welches für eine extrazellulare Domäne des CO-029-Antigens codiert, wobei die Aminosäuresequenz des Antigens in der Fig. 3 dargestellt ist.

9. Verfahren zur Herstellung eines Polypeptids, welches aus einer extrazellulären Domäne des CO-029-Antigens besteht, wobei die Ammosäuresequenz des Antigens in der Fig. 3 dargestellt ist.

10. Verfahren nach Anspruch 9, worin die Domäne die Aminosäuren 34 bis 57 von CO-029 umfaßt.

11. Verfahren nach Anspruch 9, worin die Domäne die Aminosäuren 110 bis 205 von CO-029 umfwaßt.

12. Verfahren zur Herstellung eines Polypeptids nach Anspruch 9, welches mit dem monoklonalen Antikörper CO-029 eine Immunreaktion eingeht.

13. Verfahren nach Anspruch 9, wonn das Polypeptid kovalent an eine Aminosäuresequenz gebunden ist, welche die Sekretion des Polypeptids aus Zellen verursacht.

14. Verfahren zur Herstellung eines Präparats von CO-029-Antigen, welches frei von anderem menschlichen Protein ist, wobei das Antigen in nicht-menschlichen Zellen gebildet worden ist, welche nach dem Verfahren gemäß Anspruch 1 mit einem das Antigen codierenden DNA-Molekül transformiert worden sind.

15. Mit dem DNA-Molekül der Ansprüche 1. 6. 7 oder 8 transformierte Zellen.

16. Nicht-menschliche Zellen nach Anspruch 15.

Revendications

Revendications pour les Etats contractants suivants: AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Molécule d'ADN dépourvue d'introns codant pour un antigène associé à une tumeur, ledit antigène étant immunoréactif à l'anticorps monoclonal CO-029, pour laquelle la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

2. Molécule d'ADN dépourvue d'introns selon la revendication 1, pour laquelle l'antigène associé à une tumeur présente un poids moléculaire protéinique de 26,044.

3. Molécule d'ADN dépourvue d'introns selon l'une des revendications 1 ou 2, dont la séquence d'acides nucléiques est représentée sur la figure 3.

4. Molécule d'ADN dépourvue d'introns selon l'une quelconque des revendications précédentes, qui est liée par covalence à un vecteur qui peut se répliquer dans E. coli.

5. Molécule d'ADN dépourvue d'introns selon l'une quelconque des revendications précédentes, qui provoque l'expression dans les cellules COS d'une protéine immunoréactive avec l'anticorps monoclonal CO-O29.

6. Molécule d'ADN dépourvue d'introns consistant en une séquence d'acides nucléiques représentée sur la figure 3.

7. Molécule d'ADN dépourvue d'introns, qui s'hybride avec de l'ADN ayant la séquence représentée sur la figure 3.

8. Molécule d'ADN dépourvue d'introns codant pour un domaine extra-cellulaire de l'antigène CO-029, pour laquelle la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

9. Polypeptide consistant en un domaine extra-cellulaire de l'antigène CO-029, pour lequel la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

10. Polypeptide selon la revendication 9, pour lequel le domaine comprend les acides aminés 34 à 57 de CO-029.

11. Polypeptide selon la revendication 9, pour lequel le domaine comprend les acides aminés 110 à 205 de CO-029.

12. Polypeptide selon la revendication 9, qui est immunoréactif à l'anticorps monoclonal CO-029.

13. Polypeptide selon la revendication 9, qui est lié par covalence à une séquence d'acides aminés provoquant la sécrétion dudit polypeptide par des cellules.

14. Préparation d'antigène CO-029 libre de toute autre protéine humaine, dans laquelle l'antigène est fabriqué dans des cellules non humaines qui ont été transformées avec une molécule d'ADN selon la revendication 1 codant pour ledit antigène.

15. Cellules transformées avec la molécule d'ADN selon l'une des revendications 1, 6, 7 ou 8.

16. Cellules selon la revendication 15, qui sont non humaines.

Revendications pour l'Etat Contractant suivant: ES

1. Procédé de production d'un antigène associé à une tumeur, comprenant l'utilisation d'une molécule d'ADN dépourvue d'introns codant pour un antigène associé à une tumeur, ledit antigène étant immunoréactif à l'anticorps monoclonal CO-029, dans lequel la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

2. Procédé selon la revendication 1, dans lequel l'antigène associé à une tumeur présente un poids moléculaire protéinique de 26,044.

3. Procédé selon l'une des revendications 1 ou 2, dans lequel la séquence d'acides nucléiques de ladite molécule d'ADN est représentée sur la figure 3.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel la molécule d'ADN dépourvue d'introns est liée par covalence à un vecteur qui peut se répliquer dans E. coli.

5. Procédé selon l'une quelconque des revendications précédentes, dans lequel la molécule d'ADN dépourvue d'introns provoque l'expression dans les cellules COS d'une protéine immunoréactive avec l'anticorps monoclonal CO-029.

6. Procédé comprenant l'utilisation d'une molécule d'ADN dépourvue d'introns consistant en une séquence d'acides nucléiques représentée sur la figure 3.

7. Procédé comprenant l'utilisation d'une molécule d'ADN dépourvue d'introns, qui s'hybride avec de l'ADN ayant la séquence représentée sur la figure 3.

8. Procédé comprenant l'utilisation d'une molécule d'ADN dépourvue d'introns codant pour un domaine extra-cellulaire de l'antigène CO-029, dans lequel la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

9. Procédé de production d'un polypeptide consistant en un domaine extra-cellulaire de l'antigène CO-029, dans lequel la séquence d'acides aminés dudit antigène est représentée sur la figure 3.

10. Procédé selon la revendication 9, dans lequel le domaine comprend les acides aminés 34 à 57 de CO-029.

11. Procédé selon la revendication 9, dans lequel le domaine comprend les acides aminés 110 à 205 de

CO-029.

12. Procédé de production d'un polypeptide selon la revendication 9, qui est immunoréactif à l'anticorps monoclonal CO-029.

13. Procédé selon la revendication 9, dans lequel le polypeptide est lié par covalence à une séquence d'acides aminés provoquant la sécrétion dudit polypeptide par des cellules.

14. Procédé de production d'une préparation d'antigène CO-O29 libre de toute autre protéine humaine, dans lequel l'antigène est fabriqué dans des cellules non humaines qui ont été transformées avec une molécule d'ADN selon la revendication 1 codant pour ledit antigène.

15. Cellules transformées avec la molécule d'ADN selon l'une des revendications 1, 6, 7 ou 8.

16. Cellules selon la revendication 15, qui sont non humaines.

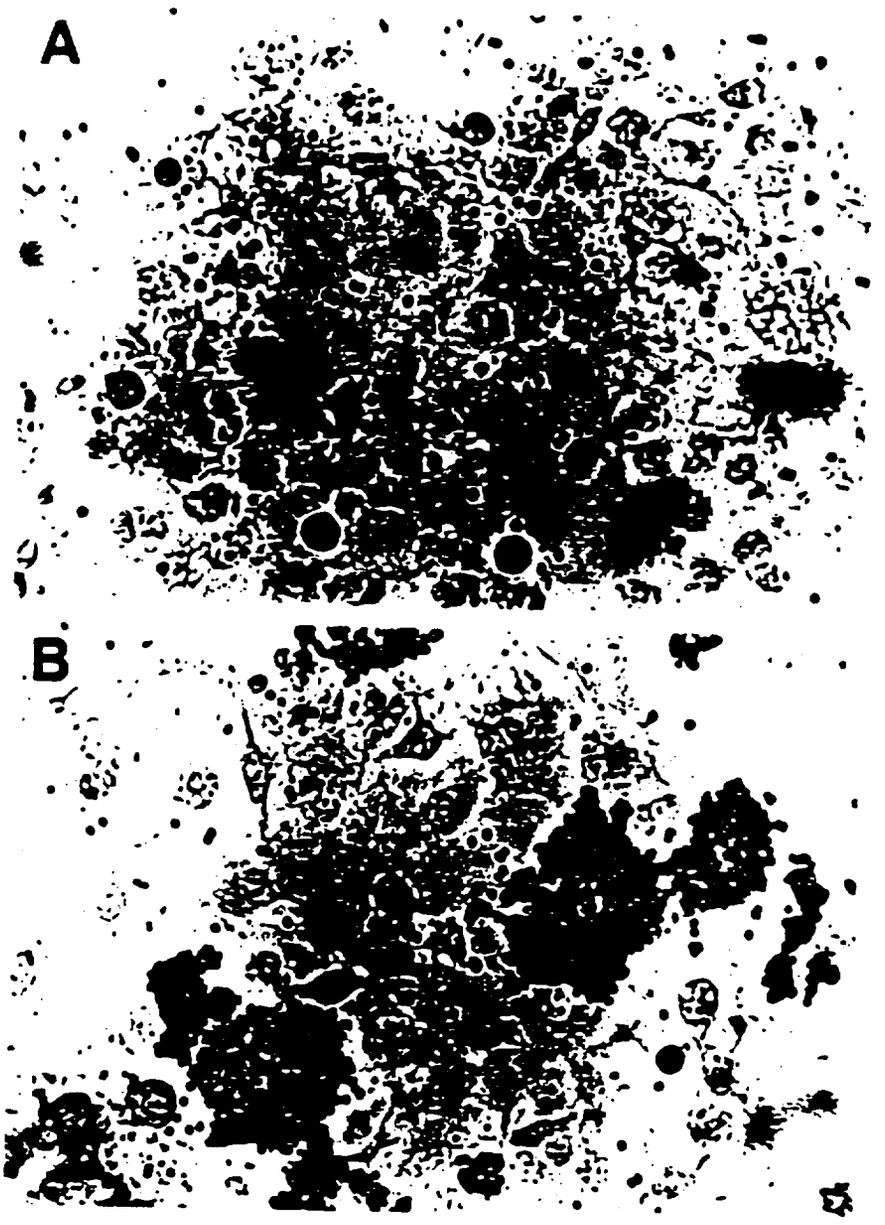
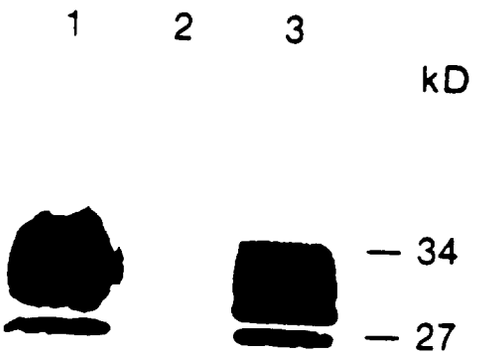


Figure 1


1
2
3
kD
34
27

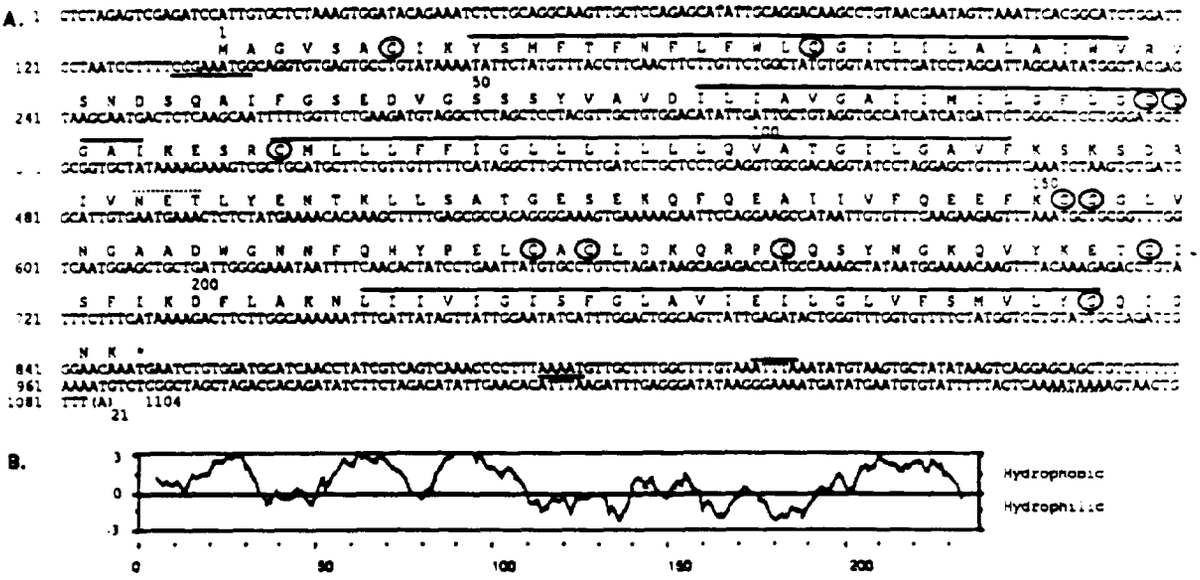


Figure 3

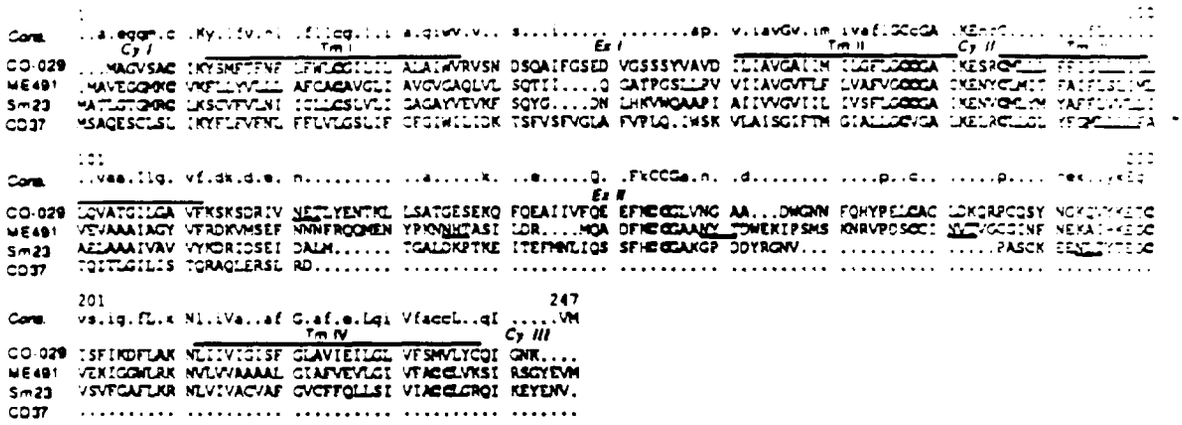


Figure 4

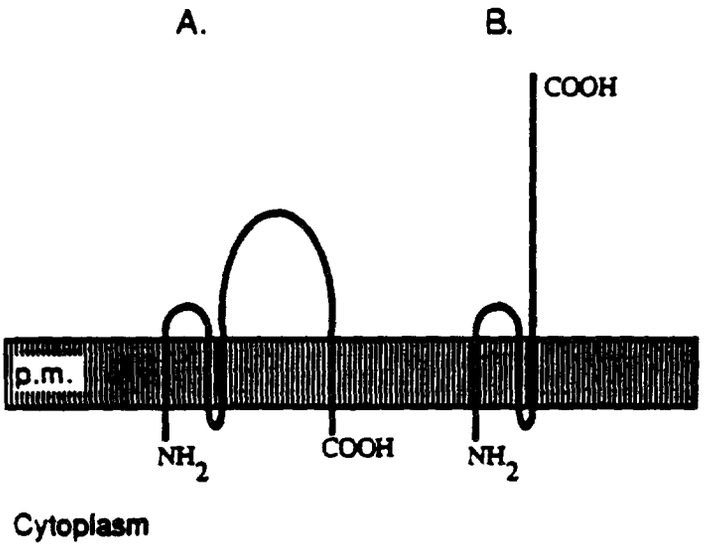
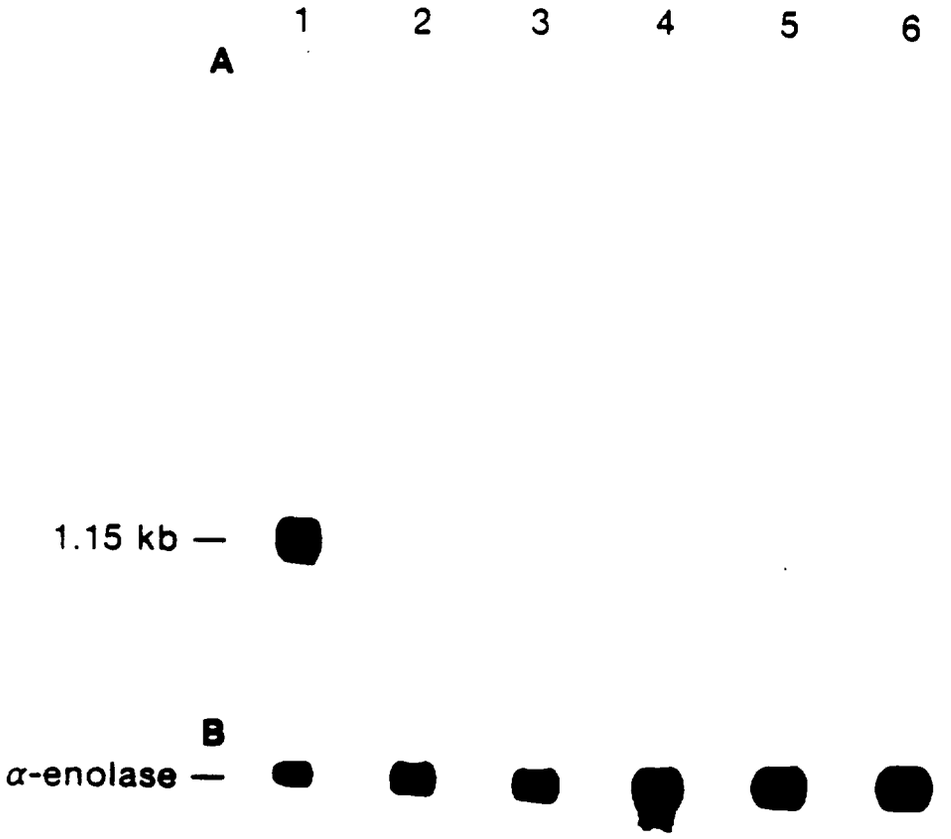


Figure 5


A
1
2
3
4
5
6
1.15 kb —
B
α-enolase —

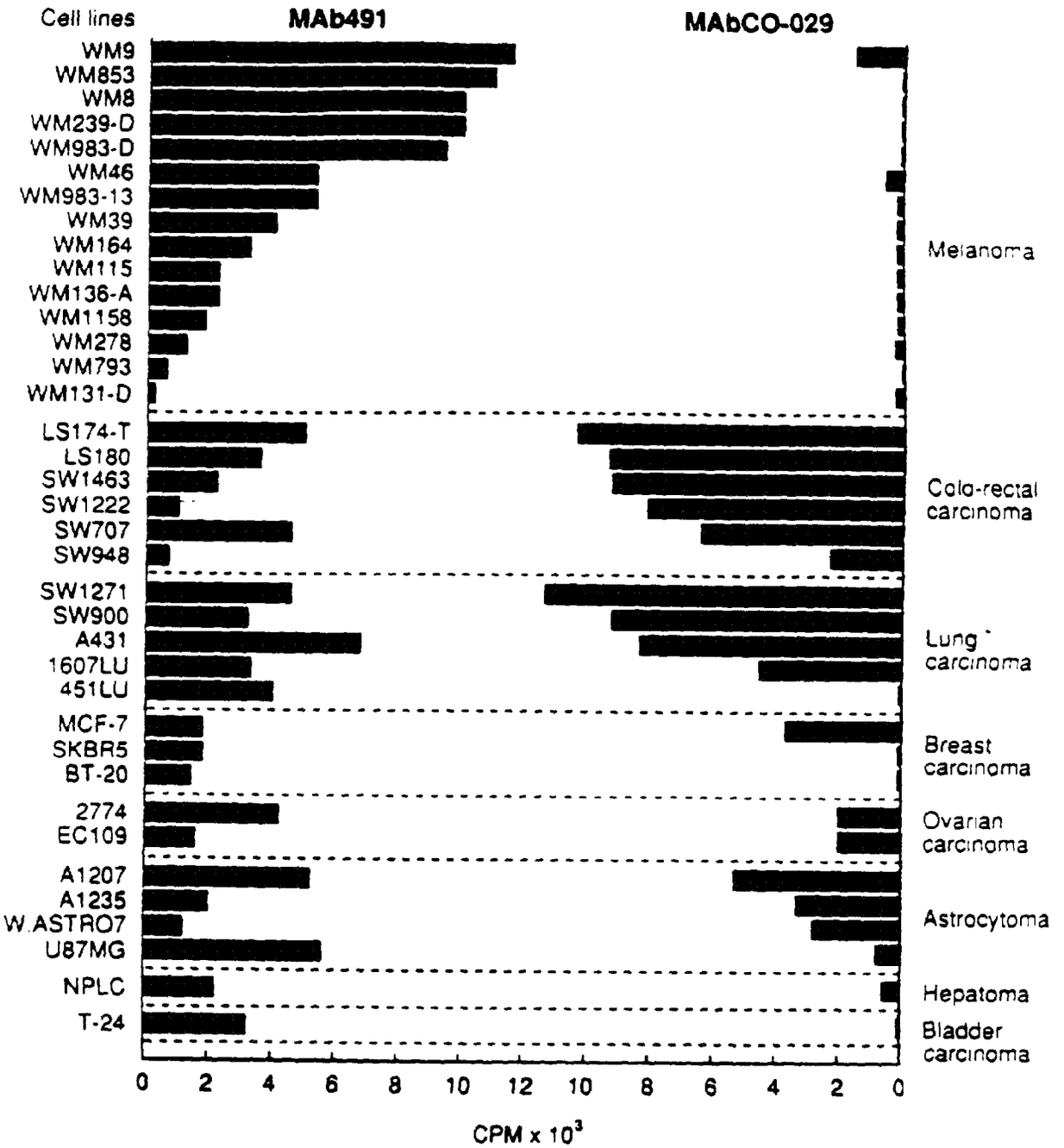

Cell lines
MAb491
MAbCO-029
WM9
WM853
WM8
WM239-D
WM983-D
WM46
WM983-13
WM39
WM164
WM115
WM136-A
WM1158
WM278
WM793
WM131-D
LS174-T
LS180
SW1463
SW1222
SW707
SW948
SW1271
SW900
A431
1607LU
451LU
MCF-7
SKBR5
BT-20
2774
EC109
A1207
A1235
W.ASTRO7
U87MG
NPLC
T-24
Melanoma
Colo-rectal carcinoma
Lung carcinoma
Breast carcinoma
Ovarian carcinoma
Astrocytoma
Hepatoma
Bladder carcinoma
0 2 4 6 8 10 12 10 8 6 4 2 0
CPM x 10³

Figure 7